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Microencapsulation of β-galactosidase with different biopolymers by a spray-drying process

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Abstract

The aim of this work was to investigate the possibility of producing microparticles containing β-galactosidase, using different biopolymers (arabic gum, chitosan, modified chitosan, calcium alginate and sodium alginate) as encapsulating agents by a spray-drying process. This study focused on the enzyme β-galactosidase, due to its importance in health and in food processing. Encapsulation of β-galactosidase can increase the applicability of this enzyme in different processes and applications. A series of β-galactosidase microparticles were prepared, and their physicochemical structures were analysed by laser granulometry analysis, zeta potential analysis, and by scanning electron microscopy (SEM). Microparticles with a mean diameter around 3 μm have been observed, for all the biopolymers tested.The microparticles formed with chitosan or arabic gum presented a very rough surface; on the other hand, the particles formed with calcium or sodium alginate or modified chitosan presented a very smooth surface.The activity of the enzyme was studied by spectrophotometric methods using the substrate ONPG (O-Nitrophenyl-β,D-galactopyranoside). The microencapsulated β-galactosidase activity decreases with all the biopolymers. The relative enzyme activity is 37, 20, 20 and 13%, for arabic gum, modified chitosan, calcium alginate and sodium alginate, respectively, when compared with the free enzyme activity. The enzyme microparticles formed with arabic gum shows the smallest decrease of Vmax, followed by the calcium alginate, sodium alginate, and modified chitosan.

Keywords: β-Galactosidase, Biopolymers, Immobilization, Microencapsulation, Spray-drying.

1. Introduction

Microencapsulation of biomolecules has become a challenging approach to design new materials used in food and pharmaceutical industries to improve stability, delivery and to control the release of encapsulated species. The preparation of enzyme microcapsules requires extremely well controlled conditions, for example the encapsulating agent should provide a suitable environment for the microencapsulation of bioactive compound (Alexakis et al., 1995; Desai & Park, 2005; Gouin, 2004).

Nowadays, biopolymers like chitosan (Biró, Németh, Sisak, Feczkó, & Gyenis, 2008), alginate (Haider & Husain, 2009), arabic gum (Lambert, Weinbreck, & Kleerebezem, 2008) have attracted interest as a matrixes for the immobilization or controlled release of innumerous enzymes and have been applied in the pharmaceutical, food, biomedical, chemical, and waste-treatment industries (Ghanem & Skonberg, 2002).

Chitosan has been widely used for enzyme immobilization in food processing, owing to its low cost, lack of toxicity and high protein affinity (Biró et al., 2008; Cheng, Duan, & Sheu, 2006; Ju et al., 2012; Liu et al., 2011; Nunthanid et al., 2008). Chitosan is obtained by partial alkaline deacetylation of chitin, which is the second most abundant natural polymer in nature after cellulose and is found in the structure of a wide number of invertebrates (crustaceans, exoskeleton insects, cuticles) among others (Kumar, 2000). Although chitosan is an attractive biomacromolecule, it is a water-insoluble material, only soluble in acidic solutions due to its rigid crystalline structure and deacetylation degree, thus limiting its application as bioactive agents such as drug carriers (Sashiwa, Kawasaki, & Nakayama, 2002; Zhang, Wu, Tao, Zang, & Su, 2010). It is possible to modify the structure in order to produce an easily soluble chitosan in neutral aqueous solutions. Water soluble chitosan can be useful for drug carriers and for food industrial applications (Estevinho, Damas, Martins, & Rocha, 2012; Estevinho, Rocha, Santos, & Alves, 2013).

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Other polymers extensively used as biomaterial are the alginates, which are natural, linear, unbranched polysaccharides containing 1,4´-linked beta-D-mannuronic and alpha-L-guluronic acid residues (Möbus, Siepmann, & Bodmeier, 2012). Alginates are derived from brown seaweeds such as *Laminiaria digitata* and *L. hyperboria* and they share a chemical structure similar to polysaccharide components of the extracellular matrix.

The alginates are currently processed as high purity and low toxicity biocompatible polymers (Mata, Igartua, Patarroyo, Pedraz, & Hernández, 2011; Yoo, Song, Chang, & Lee, 2006). Alginates have been applied for several applications related to microencapsulation and controlled release delivery systems, namely for protein (George & Abraham, 2006; Möbus et al., 2012) and enzymes (Dashevsky, 1998; Haider & Husain, 2008). Alginate gel structure is relatively stable at acidic pH, but it is easily swollen and disintegrated under mild alkali conditions (Yoo et al., 2006). Alginates are able to form water-insoluble gels upon cross-linking with divalent cations (e.g., Ca^{2+}, Zn^{2+}). (Moebus, Siepmann, & Bodmeier, 2012).

The arabic gum is a polymer consisting of D-glucuronic acid, L-rhamnose, D-galactose, and L-arabinose, with approximately 2% protein (Dickinson, 2003). Arabic gum was used as a matrix to encapsulate several enzymes, such as endoglucanase from *Thermomonospora* sp. This encapsulated enzyme retained complete biocatalytic activity and exhibited a shift in the optimum temperature [50–55 °C] and considerable increase in pH and temperature stabilities as compared to the free enzyme (Ramakrishnan, Pandit, Badgujar, Bhaskar, & Rao, 2007). In addition microparticles with arabic gum were prepared by a spray-drying method with Lipase from *Yarrowia lipolytica* (Alloue, Destain, Amighi, & Thonart, 2007).

Spray-drying technique is one of the several methods for enzyme microencapsulation. In this method, protein solution or emulsion is sprayed into the air by atomization, usually

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at elevated temperatures to evaporate the solvent. The properties of final microspheres depend on the nature of the feeding flow as well as the operating parameters such as flow rate and inlet temperature. The main advantages of spray-drying include the easy control of microsphere properties by changing the operational parameters, and the convenience in scale-up (Ye, Kim, & Park, 2010).

An important concern in the production of commercial proteins and enzymes is the preservation of their properties, namely stability and activity, during storage. Water facilitates or mediates a variety of physical and chemical degradations. Consequently, dry solid formulations of immobilized enzymes are often developed to provide an acceptable protein shelf life (Alloue et al., 2007; Namaldi, Çalik, & Uludag, 2006). The spray-drying process has proven to be an efficient method to dehydrate and to preserve lipase from *Y. lipolytica* in the presence of additives (Alloue et al., 2007; Namaldi et al., 2006).

In the present work, the authors propose the use of different biopolymers, as encapsulating agents for β-galactosidase, by a spray-drying process. β-galactosidase is one of the most important enzymes used in food and pharmaceutical industry. A significant percentage of the world population suffers from lactose intolerance or has difficulty in consuming milk and dairy products caused by the lack of β-galactosidase activity (Grosová, Rosenberg, & Rebroš, 2008; Panesar, Kumari, & Panesar, 2010). β-galactosidase can be used in a number of ways to hydrolyze lactose in milk and whey/whey permeate (Ansari & Husain, 2012; Singh & Singh, 2012; Wentworth, Skonberg, Darrel, & Ghanem, 2004). The enzyme β-galactosidase can also be used in transglycosylation of lactose to synthesize galacto-oligosaccharides (GOS), which promotes the growth of bifido-bacteria in vivo (Cheng et al., 2006; Pan et al., 2009; Shin, Park, & Yang, 1998). So, the major goal of this work was to create and compare different β-galactosidase microparticles systems, and to evaluate the different biopolymers as enzyme encapsulating materials. In

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particular, the effect of the encapsulation on enzyme activity, stability of suspension, morphology and size of particles was studied. The preparation of β-galactosidase microcapsules can enable and increase the application of this enzyme in the food industry and in healthcare.

2. Material and Methods

2.1. Preparation of the solutions

β-galactosidase microparticles were formed using 5 different biopolymers: chitosan, a modified chitosan (water soluble), sodium alginate, calcium alginate and arabic gum. High purity reagents were used in all the experiments.

Chitosan of medium molecular weight, with a Brookfield viscosity of 200 mPa.s (1wt% in 1% acetic acid; 25ºC) (448877-250g) was purchased from Aldrich (Germany). Water soluble chitosan (pharmaceutical grade water soluble chitosan) was obtained from China Eastar Group (Dong Chen) Co., Ltd ((Batch no. SH20091010). Water soluble chitosan was produced by carboxylation and had a deacetylation degree of 96.5% and a viscosity of 5 mPa.s (1%; 25 ºC). Sodium alginate (alginic acid, sodium salt) (180947-100g) was from Aldrich (USA) and the calcium alginate was prepared from sodium alginate with calcium chloride (F1435283624 – 1.02083.1000) from Merck (Darmstadt, Germany). Arabic gum (ref.51201 – 1315371 – 24606P04) was from Fluka (Germany).

Sodium tripolyphosphate (Cat:23,850-3 – lot:07027HI-416-25g) was from Sigma Aldrich (USA) and the acetic acid (glacial) (k40866663009 – 1.00063.2511) was from Merck (Darmstadt, Germany).

All the solutions were prepared at room temperature. The chitosan solution was prepared with a concentration of 1% (w/V) in an acetic acid solution (1% (V/V)) and with 2 hours agitation at 1200 rpm (magnetic agitator – MS-H-Pro, Scansci). The solutions of water

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soluble chitosan 1% (m/V), sodium alginate 1% (w/V) and Arabic gum 1% (w/V) were prepared with deionised water and with 2 hours agitation at 1200 rpm. Calcium alginate solution was prepared from a sodium alginate solution (1% (w/V)) adding a calcium chloride solution with the concentration of 1% (w/V).

β-galactosidase enzyme (*Escherichia coli*) from Calbiochem (Cat 345,788 ; EC number: 3.2.1.23) with a specific activity = 955 U/mg protein and BSA (bovine serum albumin) purchased from Sigma Aldrich (A7906-100g) were used in the preparation of the enzyme solution. A stock solution composed by β-galactosidase (0.1 mg/ml) and BSA (1 mg/ml) was prepared using a 0.08 M phosphate buffer at pH 7.7. BSA is used to stabilize enzymes and to prevent termodegradation, adhesion of the enzyme to reaction tubes, pipet tips, and other vessels (Chang & Mahoney, 1995).

2.2. Experimental conditions – Spray-drying process

Spray-drying was performed using a spray-dryer BÜCHI B-290 advanced (Flawil, Switzerland) with a standard 0.5 mm nozzle. The same procedure was followed for all the types of microparticles prepared. The solution containing the enzyme was added and mixed with each one of the biopolymers aqueous solutions at constant agitation speed of 1200 rpm, during 10 min at room temperature. The concentration of the enzyme in the fed solution to the spray-dryer was 0.4 % (w/w).

The enzyme-biopolymer solutions were spray-dried, separately, under the following conditions: solution and air flow rates, air pressure and inlet temperature were set at 4 ml/min (15%), 32 m^3/h (80%), 6.5 bar and 115 ºC, respectively. The outlet temperature, a consequence of the other experimental conditions and of the solution properties, was around 57 ºC (Table 1). The operating conditions have been selected considering

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preliminary studies and the works of other authors (Estevinho et al., 2012; Samborska, Witrowa-Rajchert, & Gonçalves, 2005).

2.3. SEM characterization

Structural analysis of the surface of the particles was performed by scanning electron microscopy (Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M). The surface structure of the particles was observed by SEM after sample preparation by pulverization of gold in a Jeol JFC 100 apparatus at Centro de Materiais da Universidade do Porto (CEMUP).

2.4. Particle size distribution

The size distribution of the microparticles was assessed by laser granulometry using a Coulter -LS 230 Particle Size Analyser (Miami, USA). The particles were characterized by number and volume average. The results were obtained as an average of three 60-seconds runs. To avoid the particles agglomeration, ethanol was used as a dispersant and the samples were ultrasound-irradiated.

2.5. Zeta potential

The zeta potential is representative of the particle charge. The zeta potential of the particles was measured by zeta sizer - Nano ZS (MALVERN Instruments – United Kingdom). The zeta potential for all the samples was evaluated in deionized water (pH around 7), and every measured value is an average of 12 runs. Assays were made in triplicate.

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2.6. β-galactosidase activity

The evaluation of the enzyme activity was based on absorbance values, read in an UV-Visible spectrophotometer (UV-1700 - PharmaSpec - SHIMADZU) at 420 nm and at room temperature. The enzyme activity was tested with the substrate ONPG (O-nitrophenyl β, D-galactopyranoside), purchased from Merck (ref 8.41747.0001). A stock solution of ONPG was prepared with a concentration of 2.25 mM. To test the activity of β-galactosidase, the enzyme was exposed to different ONPG concentrations (0.225 mM, 0.198 mM, 0.180 mM, 0.135 mM, 0.090 mM, 0.068 mM, 0.045 mM and 0.018 mM).The enzymatic reaction was started by adding the enzyme solution (either in the free form, or in the microencapsulated form) to the cuvette containing the buffer solution (phosphate buffer at pH 7.7 (0.08 M)) and the substrate. The enzyme concentration, in the microencapsulated enzyme assays, is estimated by mass balance (considering the initial concentration of the enzyme in the solution and assuming that the mass proportion of the enzyme/encapsulating agent was kept constant during the spray-drying process) and corresponds to the same value used in the free enzyme assays and equal to 0.001 mg/ml. The cuvette was stirred for 20 s. The formation of an orange coloured product (O-nitrophenol (ONP)) that absorbs at 420 nm allows the monitoring of the enzymatic reaction. The value of the absorbance was recorded at time intervals of 30 s. For each β-galactosidase reaction curve the initial velocity was calculated, according to the methodology described in Switzer & Garrity, (1999). A linear regression method (Lineweaver-Burk method), was performed to determine the Michaelis-Menten parameters.

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3. Results and Discussion

The microencapsulation process has been performed in order to obtain microparticles with different formulations. With a product yield (quantity of powder recovered reported to the quantity of raw materials used) ranging from 36 to 59% (arabic gum – 56%, chitosan – 48%, modified chitosan – 59%, calcium alginate – 36%, sodium alginate – 37%), the prepared microparticles were characterized and the enzymatic activity was evaluated.

3.1. Microparticles characterization

3.1.1. Scanning electron microscopy (SEM)

Spherical microparticles, with regular shape, were produced in all the cases (Fig. 1). The surface of the microparticles presented different textural characteristics. In the case of the particles formed with chitosan or arabic gum the surface was very rough. The particles formed with calcium or sodium alginate or modified chitosan presented a very smooth surface. In SEM images, the size of the microparticles that contained enzyme appears to be similar to the size of the microparticles alone.

3.1.2. Particle size

All the microparticles formed have a mean diameter around 3 μm (Fig. 1). This result was confirmed by analyzing the SEM images and by laser granulometry using a Coulter -LS 230 Particle Size Analyser (Miami, USA). As example, it is presented in Fig. 2, the size distribution for the case of the β-galactosidase microparticles produced with modified chitosan. For all the samples the average value (from triplicates) of the particle size was

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around 3 μm. The size of the microparticles without enzyme and β-galactosidase microparticles with the different biopolymers was assessed, and it can be concluded that the presence of the enzyme did not influence significantly the size of the microparticles.

3.1.3. Zeta potential

The stability of many colloidal systems is directly related to the magnitude of their zeta potential. In general, if the value of the particle´s zeta potential is large (positive or negative), the colloidal system will be stable. Commonly it is accepted that a zeta potential higher than 30-40 mV (negative or positive) is indicative of a stable colloidal system. Conversely, if the particle´s zeta potential is relatively small, the colloid system will tend to agglomerate. Table 2 shows zeta potential values measured in water for the microparticles formed with different biopolymers. The value of the zeta potential increases in the following order: arabic gum, chitosan, modified chitosan, calcium alginate and finally sodium alginate. Therefore, the more stable systems are obtained with the sodium alginate particles. If one compares the values of zeta potential with and without enzyme, it seems that the behaviour is not the same for the different biopolymers. Neverthless these differences are not significant as Table 2 shows. In all the cases, the value obtained is associated to a stable colloidal system.

3.2. Enzymatic activity of microencapsulated β-galactosidase

With the present work it is intended to compare the behaviour of the β-galactosidase enzyme (free or microencapsulated) with different biopolymers. In Fig. 3, the evolution of the enzymatic reaction during the reaction time for free and microencapsulated enzyme is depicted. The concentration of substrate of 0.225 mM was selected to show the effect of the microencapsulation on the enzyme activity. It can be observed that the rate of

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formation of the coloured compound ONP is lower for the microencapsulated enzymes and the lowest rate is observed for the microencapsulated enzyme with chitosan. Chitosan is a biocompatible polymer and has been used in many applications including drug delivery systems however, has a big disadvantage. Chitosan solubility is limited in water and neutral pH solutions. Although it is soluble at acid pH, the low pH tends to denature most proteins and cells (Taqieddin & Amiji, 2004).

From Fig. 3 it can also be observed that the maximum velocity does not happen at the beginning of the reaction. This delay is associated to the microencapsulated form. On the other hand the type of encapsulating agent tested allowed different types of enzyme microparticles. A more permanent immobilization of the enzyme is obtained with calcium and sodium alginate and with chitosan. The microparticles suffer a process of swelling in water, with the time, a normal process of core compounds release. This process is faster in the case of arabic gum and the modified chitosan. So, with arabic gum and modified chitosan the enzyme is completely released in the solution after 10-15 minutes.

Alginate gel structure was easily swollen and disintegrated under mild alkali conditions (Yoo et al., 2006) However, it is able to form water-insoluble gels upon cross-linking with divalent cations (e.g., Ca^{2+}, Zn^{2+}). In the case of the chitosan, it is low soluble in water.

As described by other authors, the type of biopolymer used induces the type of microparticles porosity and shell resistance, which affects the diffusion of substrate to and from the microparticle (González Siso et al., 1997). Also, the molecular weight of the substrate is an important parameter to consider. If the substrate has a high molecular weight, it might not enter so easily in the microparticles; in that case, only the protein immobilized in the surface of the microparticle is active (González Siso et al., 1997).

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The velocity of conversion and subsequently the activity of the enzyme is influenced by diffusional mechanisms through the microparticle and, eventually, by conformational changes as described by other authors (González Siso et al., 1997; Haider & Husain, 2008). The highest activity was obtained for the microparticles with arabic gum, modified chitosan (at pH 8) and calcium alginate. The relative enzyme activity is 37, 20, 20 and 13%, for arabic gum, modified chitosan (pH 8), calcium alginate and sodium alginate, respectively, when compared with the free enzyme activity. The relative enzyme activity was determined dividing the activity in each case by the highest enzyme activity (free enzyme).

The evolution of the enzymatic reaction for different substrate concentrations of ONPG have been tested between 0.018 mM and 0.225 mM for all the β-galactosidase formulations. For each β-galactosidase reaction curve the initial velocity was calculated, and the Lineweaver-Burk linearization was performed to determine the Michaelis-Menten parameters (Fig. 4). The fitting of the experimental data to the Lineweaver-Burk representation shows high correlation coefficients (>0.97) for all the enzyme formulations. The Michaelis-Menten parameters were determined for the 4 biopolymers and are presented in Table 3.

The *Vmax* parameter, representing the maximum reaction rate at a given enzyme concentration, decreased after immobilization thus confirming what was observed by Haider & Husain (2009). Some active centers are likely to be blocked after immobilization, which reduces the reaction rate, causing the decrease of the maximum reaction velocity. This decrease is more significant in the cases of the assays with sodium alginate and modified chitosan. The enzyme microparticles formed with arabic gum had the smallest decrease of *Vmax.* On the other hand, calcium alginate has a *Vmax* almost two times higher than the sodium alginate.

The *Km* parameter is associated to the affinity between the enzyme and the substrate. A smaller *Km* value indicates a greater affinity between the enzyme and substrate, and it means that the reaction rate reaches *Vmax* faster. In the case of the β-galactosidase microparticles the *Km* parameter increases in all the formulations except in the case of the modified chitosan. The decrease of *Km* with modified chitosan was already discussed by Estevinho et al. (2012) (Estevinho et al., 2012). The increase of the *Km* parameter in calcium alginate formulation comparing with the value obtained with sodium alginate can be explained by the type of crosslinking strengths established between alginate and the calcium ion. Alginates are linear polysaccharides and are soluble in water, however they are able to form water-insoluble gels by crosslinking with divalent cations like Ca^{2+} (George & Abraham, 2006; Moebus et al., 2012). Sodium alginate is able to form a gel structure, so called "egg-box model", when sodium ion is replaced by calcium (Yoo et al., 2006). Accordingly, the microparticles formed with calcium alginate tend to be more resistant and compact, reducing the affinity between the enzyme and the substrate.

4. Conclusion

The present work demonstrates that microencapsulation of the β-galactosidase enzyme using different biopolymers by a spray-drying technique is possible.

Differences on the morphology and size of the enzyme microparticles have been observed. The microcapsules produced were spherical and had a mean diameter around 3 μm, for all the biopolymers tested. In the case of the particles formed with chitosan or arabic gum the surface was very rough; on the other hand, the particles formed with calcium or sodium alginate or modified chitosan presented a very smooth surface. The value of the potential zeta suggested that the microparticles were stable.

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The type of biopolymer used induces the type of microparticles, shell resistance, which affects the diffusion of substrate and products to and from the microparticle. Kinetic data show differences on β-galactosidase activity after encapsulation, depending on the type of matrix used. The enzyme microparticles formed with arabic gum presented the smallest decrease of enzyme activity, followed by the calcium alginate, sodium alginate and modified chitosan.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Table Captions

Table 1: Inlet (Tin) and outlet (Tout) temperatures in the spray-dryer for the formation of microparticles (without enzyme) and of β-galactosidase microparticles with different biopolymers: arabic gum, chitosan, modified chitosan, calcium alginate and sodium alginate.

Table 2: Zeta potential of the microparticles without enzyme and β-galactosidase microparticles with different biopolymers: arabic gum, chitosan, modified chitosan, calcium alginate and sodium alginate. Assays made in triplicates.

Table 3: Michaelis-Menten parameters (Km and Vmax), for the different assays with free and microencapsulated β-galactosidase. Confidence intervals at 95% of Student´s t distribution.

Figure Captions

Figure 1: SEM images of the microparticles without enzyme and β-galactosidase microparticles with different biopolymers: arabic gum, chitosan, modified chitosan, calcium alginate and sodium alginate. Amplified 25000 times, beam intensity (HV) 1500 kV, distance between the sample and the lens (WD) less than 10 mm.

Figure 2: Size distribution of β-galactosidase microparticles produced with modified chitosan.

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Figure 3: Evolution of the relative velocity of ONPG conversion in ONP for free and microencapsulated enzyme with different biopolymers (arabic gum, calcium alginate, sodium alginate, chitosan and a modified chitosan (water soluble)).

Figure 4: Lineweaver-Burk representation for free and microencapsulated β-galactosidase.

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503 Table 1: Inlet (Tin) and outlet (Tout) temperatures in the spray-dryer for the formation of
504 microparticles (without enzyme) and of β-galactosidase microparticles with different
505 biopolymers: arabic gum, chitosan, modified chitosan, calcium alginate and sodium
506 alginate.

β-galactosidase microparticles	**Without Enzyme**		**With Enzyme**	
	Tin (ºC)	Tout (ºC)	Tin (ºC)	Tout (ºC)
Arabic gum	115	53	115	56
Chitosan	115	58	115	58
Modified chitosan (water soluble)	115	54	115	54
Calcium alginate	115	56	115	57
Sodium alginate	115	60	115	61

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509 Table 2: Zeta potential of the microparticles without enzyme and β-galactosidase
510 microparticles with different biopolymers: arabic gum, chitosan, modified chitosan,
511 calcium alginate and sodium alginate. Assays made in triplicates.

Microparticles	**Without Enzyme (mV)**	**With Enzyme (mV)**
Arabic gum	-31.7 ± 3.8	-28.4 ± 4.8
Chitosan	49.4 ± 4.5	50.7 ± 3.3
Modified chitosan (water soluble)	-53.0 ± 6.1	-47.4 ± 7.0
Calcium alginate	-65.4 ± 5.1	-63.5 ± 5.6
Sodium alginate	-68.6 ± 6.1	-69.3 ± 7.1

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515 Table 3: Michaelis-Menten parameters (Km and Vmax), for the different assays with free
516 and microencapsulated β-galactosidase. Confidence intervals at 95% of Student´s t
517 distribution.

Parameters of Michaelis-Menten	**Free Enzyme**	**Arabic Gum**	**Calcium Alginate**	**Sodium Alginate**	**Modified Chitosan**
Km (mM)	0.47 ± 0.22	0.52 ± 0.18	0.77 ± 0.34	0.44 ± 0.11	0.13 ± 0.03
Vmax (micromoles of ONPG hydrolysed/min)	0.58 ± 0.30	0.29 ± 0.13	0.20 ± 0.10	0.11 ± 0.04	0.11 ± 0.04
R^2	0.99	0.98	0.98	0.99	0.97

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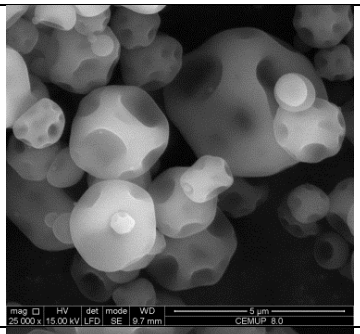
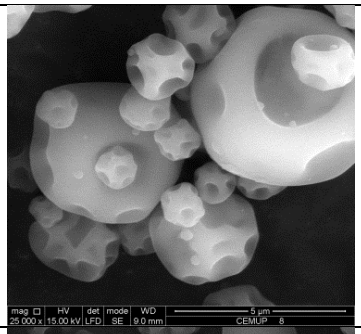
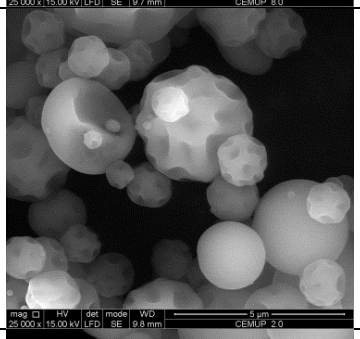
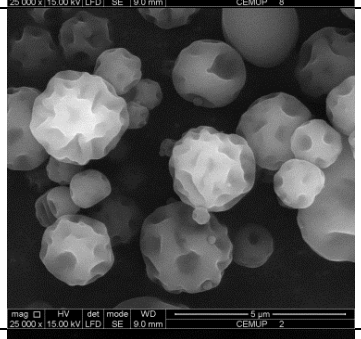
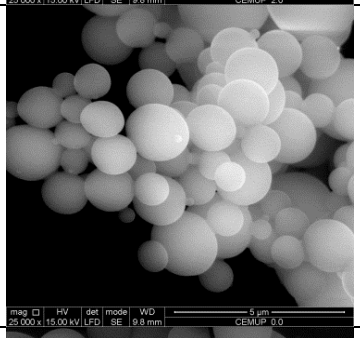
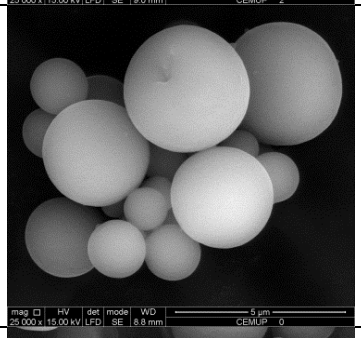
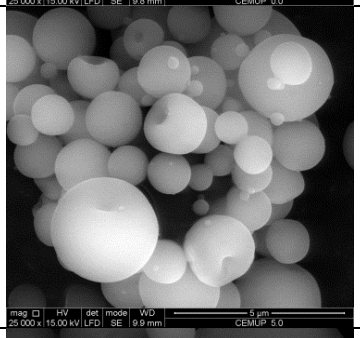
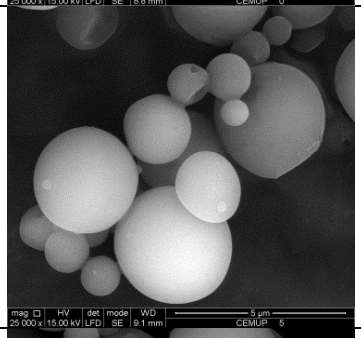
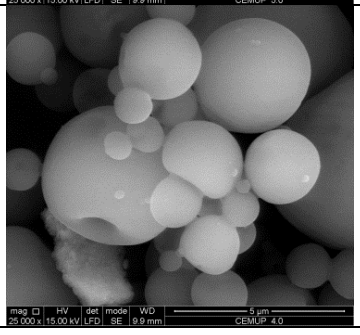
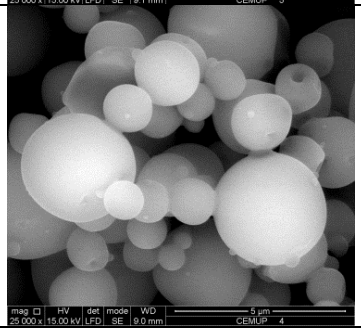
Figure 1: SEM images of the microparticles without enzyme and β-galactosidase microparticles with different biopolymers: arabic gum, chitosan, modified chitosan, calcium alginate and sodium alginate. Amplified 25000 times, beam intensity (HV) 1500 kV, distance between the sample and the lens (WD) less than 10 mm.

Biopolymers	Without Enzyme	With Enzyme
Arabic Gum		
Chitosan		
Modified Chitosan (Water Soluble)		
Calcium Alginate		
Sodium Alginate		

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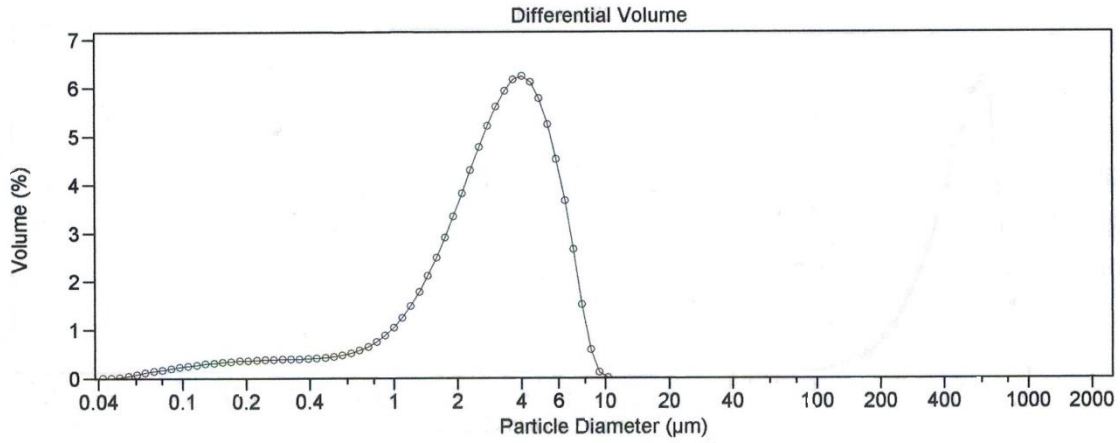

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525 Figure 2: Size distribution of β-galactosidase microparticles produced with modified

526 chitosan.

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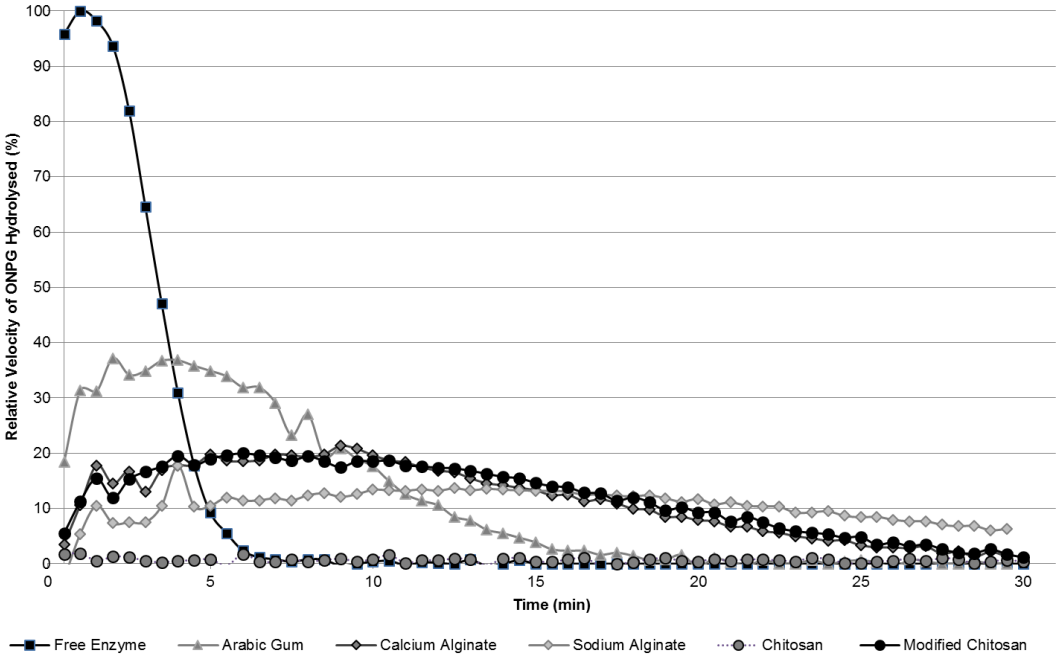

Figure 3: Evolution of the relative velocity of ONPG conversion in ONP for free and microencapsulated enzyme with different biopolymers (arabic gum, calcium alginate, sodium alginate, chitosan and a modified chitosan (water soluble)).

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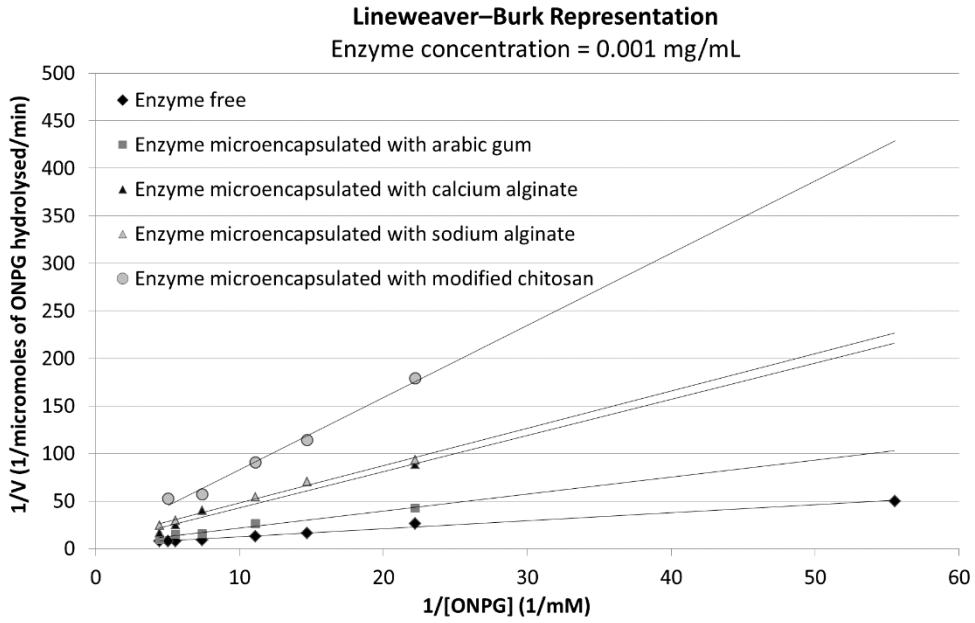

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536 Figure 4: Lineweaver-Burk representation for free and microencapsulated β-
537 galactosidase.

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